THE EFFECT OF TEMPERATURE ON GROWTH AND THE METABOLISM OF RIBONUCLEIC ACID IN RELATION TO CELL DIVISION AND CELL ELONGATION OF *PISUM SATIVUM* 'ALASKA'

By

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INTRODUCTION

In 1930 the only function one could suggest for deoxyribonucleic acid (DNA) was the role of a pH buffer inside the nucleus. During the past twenty years an understanding of the mechanism of biosynthesis of nucleic acids has become clearer, partly as a result of the elucidation of the structure of nucleic acids and partly due to improvement of chemical and physical technology. One widely accepted hypothesis is that DNA is the genetic carrier of information and ribonucleic acid (RNA) is an essential component in the expression of this information in polypeptide synthesis.

Few studies have been made concerning the relationship between growth and ribonucleic acid content in higher plants and most of the data reported have been studies on total RNA content. It is now known that different species of RNA which are functionally distinct yet closely interrelated exist in the cell. RNA plays a vital role in enzyme and protein synthesis. Many experiments have indicated that there are biochemical changes in RNA metabolism in plants associated with changes in growth and differentiation.

Temperature has long been known to affect plant growth. There are different optimal temperature ranges for different species of plants. Effects of temperature on duration of mitotic cycle, rate of cell production and rate of growth of Pisum sativum 'Alaska' root have been studied (58). Data from these studies showed that the rate of cell production was dependent on the duration of the mitotic cycle

time. The rate of root growth was dependent upon both cell production and cell expansion.

In the studies reported here the influence of temperature and time on growth and total RNA content of the root, as well as the RNA content of the root tip section, were determined in order to establish the relationship between temperature, growth and time on total RNA content. A methylated albumin column was used to separate the RNAs in an attempt to correlate the changes on various RNAs with growth rate. Both dividing and elongating cell populations were studied with the hope of gaining information on the regulating mechanism of RNAs on cell division and cell elongation. Isotope labelling experiments and sucrose density gradient analyses were carried out to study the rate of RNA synthesis and to characterize various RNA fractions which had been separated from the methylated albumin column.

REVIEW OF LITERATURE

Effect of temperature on plant growth:

Temperature has long been known to be an essential factor for maximum growth of higher plants. Temperatures at which most physiological processes occur in plants normally range from approximately 0° to 40°C (74). Effects of temperature on the plants were largely mediated by their effect on chemical reactions.

Respiration rate of plants has been shown to be affected in much the same way as any other chemical reaction, with a Q_{10} from two to three between 10° to 30°C (74). Translocation of sugar and other organic materials has also been shown to be affected by temperature (73). Early studies with pea root by Leitch in 1916 (37) pointed out that the rate of root elongation increased with the increase of temperature from -2° to 29°C. Brown (7) and Vant'Hof and Sparrow (66) obtained evidence which showed that the duration of mitotic cycles decreased as the temperature increased from 10° to 30°C. Gray and Scholes (17) discovered that in Vicia faba various parameters of cell kinetics could be determined by measuring root growth, increase in cell size, duration of mitosis and proportion of cells in mitosis. Dependency of cell size on temperature has been reported by Baldovinos (1), Brown and Rickless (5). Platenius (51) carried out an experiment to study the effects of temperature on the respiration rate of some vegetables. In 1923 Fernandes (16) studied the effects of temperature on pea root respiration and reported that in pea root a rise in temperature caused an increase in the rate

of respiration. The Q_{10} for respiration of pea root decreased from 3.4 for 0°-10°C to 2.6 for 10°-20°C and 1.6 between the temperature range of 20° to 30°C.

Equations for estimating rate of cell production and rate of root growth have been derived on the basis of duration of mitotic cycle time, number of cells in the meristematic root tip and the mitotic index (68). Results indicated that effects of temperature on root growth of Pisum sativum were primarily dependent on rate of cell production which in turn was dependent on duration of the mitotic cycle time. The effect of temperature on plant growth has been reviewed by Went (74) in the Annual Review of Plant Physiology. The influence of temperature on biological systems was discussed in detail in a symposium held at the University of Connecticut, August, 1956 (28).

Growth and differentiation of pea root:

The level at which tissues mature in the root depends to some extent on both the size and rate of growth of the root. There is some evidence which indicates that the extent and level of meristem differentiation changes when rate of growth is decreased. Fast growing roots have longer meristems and levels of differentiation are further from the apex than in slow growing roots (15).

Popham (52) estimated the rate of tissue differentiation in relation to growth rate in seedling roots of Pisum. He found that, between the fifth and twenty-first day after germination the level of differentiation was closer to the tip. In roots, most of the growth in length occurred outside the meristem in the elongating zone. This is where the most conspicuous increase in cell volume, and more especially cell length, occurs. Brown and Broadbent (6) calculated

the average cell volumes for transverse slices of Pisum root by counting the number of cells per slice. The average volume per cell was $6,800\mu^3$ over the first 400μ of the apex and $53,000\mu^3$ at $3,000\mu$ from the apex. The average volume reached a maximum of $180,000\mu^3$ at about $5,000\mu$ from the apex.

Ribonucleic acid:

There are two types of nucleic acids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). RNA differs from DNA in chemical composition, structure and configuration. Chemically the backbone of an RNA chain is similar to that of a DNA chain. Both sugar units are linked by 3',5'-phosphodiester bonds. The RNA chain, however, contains ribose instead of deoxyribose residues as does DNA. In both RNA and DNA the purine and pyrimidine bases are linked to the carbon-1 of the sugar. RNA differs from DNA in that it contains uracil instead of thymine. The RNAs from various sources differ greatly in their base ratios. The double-helical model of DNA has been proposed by Waston and Crick (70, 71), and x-ray diffraction studies support this concept.

RNA in plant cells may be classified into three groups. Soluble RNA (sRNA), ribosomal RNA (rRNA) and messenger RNA (mRNA). Soluble RNA or transfer RNA was first discovered by Hoagland, Zamecnick, and their colleagues (25, 26). The RNA component, of low molecular weight, of the soluble fraction prepared from the cytoplasmic extracts was named soluble RNA, based on the method of preparation. Soluble RNA appears to be a rod-shaped molecule with one ribonucleic acid chain containing between 60 and 80 nucleotides. This chain, with the general formula $_pG_pX\cdots\cdots\cdots\cdots\cdots{}_pY_pC_pC_pA$ (by convention the linkage G-3'-phosphate-5'A is written as G_pA) and containing methylated nucleotides

and pseudouridylic acid in the chains central region, is folded back on itself to form a base-paired structure with A-U and G-C paring between anti-parallel limbs of the chain. Two types of folding of the chain are possible, shown diagrammatically in (a) and (b) (3).

(a) (b)

Figure 1

Soluble RNA is a mixture of species of molecular weight between 25,000 and 30,000 with a sedimentation constant of about four. The end group of each polymer is -C-C-A, and in protein biosynthesis an activated amino acid is attached to the terminal-A by an ester formation between the ribose and the carboxyl group. The function of sRNA is to act as an acceptor for an activated amino acid and to transfer it to the site of protein synthesis on the messenger RNA template in polysomes, thus permitting the correct amino acid to be placed on the correct coding site (30). Lipmann and Von Ehrenstein in 1961 demonstrated that sRNA from one species may be used to synthesize a protein typical of a different species (39). Once the amino acid has been attached to a specific sRNA, the future fate of that amino acid is decided by the coding properties of the sRNA adaptor. This was first shown by Chapeville et. al. in 1962 (9). For each of the twenty naturally occurring amino acids there must be at least one, or possibly more, specific types of sRNA molecules. Another role for sRNA has been put forward by Stent and Brenner (60) and supported by Kurland and Maaloe (36), based on genetic studies of the regulation of ribosomal RNA synthesis in Escherichia coli K 12. They suggested that sRNA acts as a

repressor molecule in the regulation of sRNA synthesis in a manner analogous to that of a repressor in the general scheme of Jacob and Monod (31) for regulation of protein synthesis. They assumed that amino acids or adenylates act as inducers. At present no direct proof for this role of sRNA has been obtained.

It was reported (27, 35) that ribosomal RNA, with a molecular weight of the order of 10^6, comprises 80-90 per cent of the total cellular RNA. This fraction of RNA exists in a cell as ribonucleoprotein particles. The study of sedimentation properties of ribosomal RNAs was shown in most cases to consist of two discrete groups of molecular weight, one being around a million to a million and a half (23s), the other being close to a half million or higher (16s). Ribosomal RNA consists of a long continuous polynucleotide chain. Depending on molecular weights, these chains may contain about 1,500-2,000 or 4,000-5,000 nucleotides (59). Different sedimentation values have been assigned to various forms of RNA by different authors.

The general structure of ribosomes from animals, plants and micro-organisms is strikingly similar (55). Ribosomes are spherical structures with diameters of 200 to 300 A°. They are composed of 40 to 60 per cent protein. X-ray defraction studies of E. coli ribosomes suggested that three-fourth of their RNA has a double helical structure (80). In plant systems as reported by Ts'o (64) ribosomal particles are found largely in the cytoplasm and also in nuclear and mitochondrial fractions. The best known function of ribosome is direct participation in synthesis of proteins. Many reviews on protein synthesis have appeared recently (21, 58, 65, 72). It has been observed (55, 63) that the ribosomal fractions most active in protein synthesis

in E. coli were those with a sedimentation constant higher than 70s. These active heavy ribosomes have been named polyribosomes or polysomes. From the sedimentation constant it may be calculated that each polysome contains on the average five 76 s ribosomes. In electronmicroscopic studies Warner, Rich and Hall (69) were able to show that clusters of ribosomal particles predominated in the heavy fraction in a sucrose gradient analysis. More recently, the existence of polysome aggregates has been demonstrated in mammalian cells (20), cabbage plants (14) and maize seedlings (77). Polysomes, as we know, may be ribosomes linked together by messenger RNA. The proposed mode of action of polysomes in protein synthesis is that a single ribosome moves along the length of the messenger RNA synthesizing a polypeptide chain as they go along. When they come to the end of the messenger RNA, they fall off and release the newly formed peptide chain (30). However, this cannot as yet be taken as fully proven.

Messenger RNA (mRNA) accounts for between 5 and 10 per cent of total RNA inside E. coli cells and it makes up a similar proportion in most actively growing cells. Messenger RNA is heterogenous in molecular size, having molecular weights up to 2×10^6 with a correspondingly wide range of sedimentation constants. Messenger RNA has been defined as an RNA fraction corresponding in base composition to DNA of the particular cell under investigation. The mRNA turns over rapidly and is usually detected by selective radioactive labeling. The name and the concept of "messenger RNA" were developed by Jacob and Monod (31) in their interpretation of the mechanism of enzyme induction and repression in bacteria. The function of mRNA is to transfer the messages from DNA to the cytoplasm, and to determine the kind of

protein to be synthesized by the ribosomes. Messenger RNA does not appear to have any secondary structure. This agrees well with the supposed role of mRNA in the polysome, where a long stretched molecule in linear rather than coiled configuration is required. Present evidence (19, 22, 54) supports the Jacob and Monod (31) model of regulation of enzyme synthesis in which kinds and relative proportions of different mRNA made at any time depend on the degree to which repression blocks the transcription of the respective regions of DNA.

RNA content in root tissue:

Unlike DNA the amount of RNA in a cell is not constant, and rate of turnover of RNA is higher than that of DNA in both active and quiescent cells. Jensen (32) calculated average values for RNA per cell of Vicia faba roots. He found that at the level of the quiescent center the amount of RNA per cell was at a minimum but increased slightly in the cap and on the proximal side. A three-fold increase occurred at about 2,000μ from the tip and remained steady up to 3,000μ where the observation stopped. Jensen (33) also reported a similar situation for the roots of Allium. Heyes and Brown (23) found similar trends in RNA content per cell in slices of roots of Pisum sativum. There was a three-fold increase in average RNA values between 1,000μ and 9,000μ from the tip. Sunderland and McLeish (62) measured total amounts of RNA and DNA in segments 0-2, 2-4, 4-6, 6-8 mm from the root apices of six species of higher plants. Results indicated that progress of cell expansion from the first to the fourth section was accompanied in five species by an increase in average amounts of both RNA and DNA per cell.

RNA metabolism in relation to plant growth:

RNA's role in developing plant cells has been reviewed by Oota (47) in a recent volume of Annual Review of Plant Physiology. RNA plays a vital role in enzyme and protein synthesis. A positive correlation between total RNA content of tissue and the capacity to make protein has been shown for a variety of organisms, including higher plants (21, 29, 72). The level of ribosomal RNA was shown to be even more significately related to protein synthesis than to total RNA content (21, 29, 72). In cabbage plants (14) as well as in maize seedlings (77) protein synthesis and growth have been shown to be correlated with formation of polysomes. Woodstock and Skoog (78, 79) indicated that the rate of future elongation as well as the final overall size of corn roots are determined by the amount of RNA previously deposited in the apical portion of the root. Heyes (24) reported pea roots containing two species of RNA which differ in their extractability and base composition. He stated that maturation of roots was associated with a decrease in acid extractable RNA and an increase in alkali extractable RNA. Oota and Osawa (46) studied bean seedlings and discovered a proportionality between rate of protein synthesis and concentration of microsomal RNA in the tissues. No direct correlation was found in either the RNA content of the whole tissues or any other subcellular fractions examined. Lydon (41) studied the changes in the nucleus during cellular development in pea seedlings by isolating the nuclei from three regions of the root. RNA content of the nucleus decreased with the development of the plant, while cytoplasmic RNA increased. Lydon suggested that cellular development probably involves a change in the pattern of interaction between the nucleus and cytoplasm.

Electromicrographic studies by Lund et al. (40) showed that the dense population of ribosomes in the meristematic cells of corn root or pea embryo disappeared leaving an empty background in the cytoplasm as the cell underwent elongation.

Temperature controlled growth rate and ribonucleic acid characteristics in Mimosa epicotyl tissue have been recently reported by Brown (4). Base composition of soluble RNA was correlated with growth rates, particularly with respect to the quantities of guanine and uracil. These results suggested that there is a relationship between environmental conditions and differential synthesis of soluble RNA molecules.

Changes in nucleotide content as a function of growth rate of etiolated corn seedlings has also been reported by Cherry and Hageman (10). The ratios of mono-, di- and triphosphate nucleotides to the monophosphate nucleotides as a function of growth rate were also computed. Data indicated that a gradual shift from higher energy di- and triphosphate nucleotides to the monophosphate nucleotides occurred as the corn seed germinated.

Total RNA content of carrot phloem explants grown for a two week period in three different types of liquid culture media was determined by Steward et al. (61). Results indicated that cells which expand without division remain very high in total RNA. The maximum RNA content per cell was found in cells about to divide in the presence of coconut milk. The RNA content fell steadily as cell division proceded.

The role of RNA synthesis in the mitotic cycle has been studied (53). Generally snythesis of RNA is continuous through out the

mitotic cycle from G_1 to S to G_2. Synthesis of RNA, however, is minimal when cell division occurs. Autoradiographic studies of RNA synthesis in the mitotic cycle of pea root meristem cells also showed that there was no labeling of the cell during metaphase and anaphase (67).

The regulation of RNA synthesis:

The regulation of RNA synthesis in bacteria has been recently discussed by Neidhardt (43). He stated that the formation of RNA seems to be geared in a precise and unique manner to the over-all protein synthesizing potential of the cell in its particular environment. Synthesis of rRNA is a variable fraction of the cells' total biosynthetic activity, depending on growth rate. The concentration of rRNA in a cell is a simple linear function of the over-all rate of protein synthesis during steady-state growth. The constancy of the rate of protein synthesis calculated per unit of rRNA is the prime physiological function of this intergration. The regulation seems to be achieved by a reversible inhibition of the RNA-forming machinery of the cell, and most of the evidence is consistent with a model in which amino acids reverse this inhibition, perhaps by combining with their respective sRNAs. Fewer details are known about the regulation of nonribosomal RNA synthesis, but the formation of both sRNA and, to a lesser extent, mRNA is dependent on amino acid supply. Rosset et al. (57) measured the fraction of total RNA that is sRNA at each of a number of growth rates in E. coli ML 308. Results indicated that sRNA increased with the decrease of generations per hour.

The study of nucleic acid synthesis and its regulation has only recently been attempted in a few laboratories. Most of the information has been gained from bacteria and viruses. Limited

information is available concerning higher plants (29, 45, 75, 76).

MATERIALS AND METHODS

Experiment I: Effect of temperature and time on root growth and total RNA content:

1. Growth of pea roots:

Seeds of Pisum sativum 'Alaska' were soaked in aerated distilled water for 12 hours, then germinated in vermiculite for 36 hours at 23° C in the dark. Seedlings with a primary root about 3 cm long were removed from the vermiculite and grown in Hoagland's full strength nutrient solution at different temperatures (10°, 20°, 25° and 30° C) in the dark. The rates of root growth were measured every 24 hours for 72 hours. Fresh weight and total RNA were determined. Aeration was continuous throughout the experiment.

2. Total RNA determination:

Thirty roots were collected each day from each temperature treatment. Roots were homogenized in an ice-cold omni-mixer for five minutes with 20 ml 5 per cent sucrose, 0.005 M Tris, 0.001 M $MgCl_2$, then filtered through four layers of cheesecloth and centrifuged at 1,500 x g for 30 minutes to remove cell walls and other debris. The supernatant was decanted and saved for analysis. Total RNA was determined by the method of Ogur and Rosen (45). RNA was precipitated by adding 1 N $HClO_4$ to aliquots so that a final concentration of 0.2 M acid solution resulted. Precipitants were collected by centrifuging at 15,000 x g for 15 minutes. Lipids were removed by washing twice with 2:2:1 (v/v/v) ethanol: ether: chloroform mixture. After lipids

were removed, RNA was hydrolyzed in 5 ml of 0.5 M NaOH at room temperature overnight. The protein was then removed by addition of $HClO_4$. The pH of the supernatant was adjusted to 7.0 and the optical density measured at 260 and 290mμ with a Beckman DB spectrophotometer. The extinction coefficients of the standard nucleotides at pH 7.0 were determined and used to calculate the quantity of RNA in each sample.

Experiment II: Effect of temperature and time on total RNA content and number of cells in the one centimeter root tip sections:

1. Total RNA content of the root tip sections:

Seeds were germinated and grown in the growth chamber as described in Experiment I. Thirty roots were collected each day from each temperature treatment. The first centimeter of root tip sections were homogenized and total RNA content was determined as previously described.

2. Number of cells:

Every 24 hours ten additional roots were collected and fixed in 3:1 (v/v) ethanol: acetic acid for 24 hours. The roots were then hydrolyzed in 1 N HCl at 60°C for 10 minutes and transferred to 2 ml of 5 per cent chromic acid and macerated with a syringe to form a cell suspension. Cell counts were made by using a hemacytometer.

Experiment III: Effect of temperature on various RNA species in both dividing (R_1) and elongating (R_2) root sections:

1. Extraction of RNA:

Pea seedlings grown at different temperatures (5°, 10°, 20° and 25°C) were collected 24 hours after the temperature treatment. Two sections of each root were used in these studies: (1) R_1 (0.0-0.4 cm root tip) in which most cells were undergoing rapid division and (2)

R_2 (0.4-1.4 cm) in which most cells were expanding.

Eighty sections of root from each temperature treatment were placed in 8 ml of a solution containing 0.01 M Tris, 0.06 M KCl, 0.01 M $MgCl_2$, 1 ml bentonite (40 mg/ml), 3 ml 5.5 per cent sodium lauryl sulfate and 16 ml cold phenol, saturated with 0.01 M Tris, 0.01 $MgCl_2$ and 0.06 M KCl. The sections were homogenized in an omni-mixer one minute at full speed, one minute gently and again one minute at full speed. The homogenate was centrifuged at 20,000 x g for 10 minutes. The aqueous layer was removed with a large syringe. One ml of bentonite and one volume of cold phenol were added to this aqueous layer and shaken for five minutes in an ice bath, then centrifuged at 20,000 x g for ten minutes and extracted again with one half volume of phenol. This aqueous layer was made 2 per cent in potassium acetate and two volumes of cold absolute ethanol were added to precipitate RNA. The precipitant was then collected by centrifuging at 30,000 x g for 20 minutes. The RNA was dissolved in 5-10 ml 0.05 M sodium phosphate buffer at pH 6.7 and dialyzed for 48 hours against a 0.05 M sodium phosphate buffer, pH 6.7 at 4°C. The bentonite suspension was prepared by the method of Brownhill, Jones and Stacey (8).

2. Fractionation of RNA on methylated albumin column:

(a) Preparation of methylated albumin (MA): Two and one-half grams of bovine serum albumin (fraction V) were suspended in 250 ml absolute methanol; 4.2 ml concentrated HCl added and the mixture incubated at 37° C for five days in the dark. The methylated albumin was collected by centrifuging twice with methanol, then washing the MA with anhydrous ether several times and evaporating the ether in air, yielding MA as a white powder. The removal of HCl should be completed

as rapidly as possible to prevent hydrolysis of the MA.

A 1 per cent solution of MA was made with deionized water for the preparation of the column.

(b) Preparation of methylated albumin-coated kieselguhr (MAK): A suspension of 20 grams of kieselguhr in 100 ml of 0.1 m NaCl and 0.05 M sodium phosphate buffer, pH 6.7, was boiled and cooled. This suspension was then treated with 5 ml 1 per cent MA solution, stirred and diluted with an additional 20 ml buffered saline solution. The methylated albumin-coated kieselguhr can be stored in the cold for at least two weeks.

(c) Preparation of methylated albumin column: The methylated albumin column was prepared as outlined by Mandell and Hershey (42), except that the quantity was doubled. The column was composed of four layers (Figure 2).

Figure 2

(i) One gram paper powder in 20 ml 0.1 M NaCl and 0.05 M phosphate buffer, pH 6.7.

(ii) Eight grams kieselguhr in 40 ml of 0.1 M NaCl and 0.05 M phosphate buffer, pH 6.7, boiled and cooled, to which 2 ml of 1 per cent MA were added.

(iii) Six grams kieselguhr in 40 ml of 0.1 M NaCl and 0.05 M

sodium phosphate buffer, pH 6.7, boiled and cooled, to which 10 ml of MAK were added.

(iv) One gram kiesilguhr in 20 ml of 0.1 M NaCl and phosphate buffer, pH 6.7.

A 2 x 40 cm glass column fitted with a sintered glass disk was used. The column was packed layer by layer. A final washing of the column with at least 150 ml of starting buffer was necessary before samples were added in order to obtain a good separation of RNA.

(d) Fractionation of RNA: After 48 hours of dialysis each phenol extracted sample was diluted to 40 ml with starting NaCl and phosphate buffer, pH 6.7, and added to the methylated albumin column. When the sample level nearly reached the kiesilguhr layer on top of the column, the column was attached to the NaCl and phosphate buffer gradient system. The RNA was eluted from the column with a linear gradient of buffered NaCl, obtained as follows: 400 ml of buffered NaCl solution was placed in each of the two chambers of the gradient maker. The solution in the left-hand chamber was at the desired final concentration and was gradually introduced into the right-hand chamber through a narrow tube. The right-hand chamber contained a solution at the starting concentration and a stirrer and was fitted with a tube for introducing the mixed solutions to the column. The concentration range of the NaCl solution varied with different MA preparations and was determined by trial for each preparation, but was usually about 0.1 to 1.3 M.

When elution began, air pressure at two psi was applied to the system. A fraction collector was used to collect fractions of 5 ml which were examined spectrophotometrically at 260mμ using a Beckman

model DB spectrophotometer. The total optical density of each nucleic acid peak and the per cent composition were calculated.

Experiment IV: Effect of temperature on the metabolism of various RNA species:

1. Isotope labeling experiments:

After 48 hours germination pea seedlings were transferred to a growth chamber and grown in nutrient solution at 10° and 20° C for 24 hours. The plants were then incubated in P^{32} for 15, 30, 90, or 120 minutes for labeling. After labeling the seedlings were washed several times with distilled water. The distilled water was pre-incubated in the growth chamber to eliminate the possible changes in temperature caused by washing. In the pulse labeling experiments, after exposure to P^{32}, the seedlings were washed and returned to the nutrient solution in the growth chamber. Samples were collected 0, 1, 5 and 24 hours after labeling. Nucleic acids were extracted and separated on a methylated albumin column and 2 ml aliquots from each fraction were dried in planchets and counted on a gas flow counter.

2. Sucrose density gradient experiments:

Pea seedlings were grown and collected as previously described. After 48 hours of dialysis of the phenol extracted materials, one-third of the material was used for MA column analysis as described in Experiment III, and one-third for density gradient analysis. For density gradient analysis the sample volume was adjusted to 5 ml with 0.05 M phosphate buffer, pH 6.7, and layered on top of a 5-25 per cent sucrose density gradient. All the sucrose solutions were in 10^{-3} M $MgCl_2$ and 5 x 10^{-3} Tris. The gradients were centrifuged for 14 hours at 80,524 x g in the SW 25.1 rotor of a Spinco model L ultracentrifuge.

The centrifuge fractions were recovered by puncturing the bottom of the centrifuge tube and collecting eight drop fractions ($\approx$ 1 ml). Each fraction was precipitated with two volumes of cold ethanol and centrifuged and the precipitant redissolved in 5 ml 0.05 M phosphate buffer, pH 6.7. The optical density of each fraction was measured. Two ml aliquots of each fraction were dried in planchets and counted on a gas flow counter. The results were plotted and analyzed.

RESULTS

Experiment I: Effect of temperature and time on pea root growth and total RNA content:

1. Effect of temperature on root growth:

The rate of pea root growth (cm/hr) was greatest at 20° and 25° C (Table 1). Growth at 10° C was less than one-half that at 20° and 25° C. The growth rate of roots grown at 30° C was approximately two-thirds that of the roots grown at 20° and 25° C. There was an increase in root length at each temperature with an increase in time.

The effect of temperature on the growth rate of pea roots was not correlated with the rate of cell production (Figure 3).

2. Effect of temperature and time on total RNA content:

There was no change in total RNA content in roots grown at 10° C during the first 24 hours; after that there was a slight increase (Table 2). RNA content increased linearly with time in roots grown at 20° C. There was a quadratic increase in RNA content of roots grown at 25° C, with a large increase at 72 hours. At 30° C, there was an increase in RNA content from 0 to 48 hours but only a slight increase at 72 hours. The per centage of increase in RNA content, as related to temperature and time, is shown in Table 3. The relationship between temperature, growth rate and total RNA content per root is presented in Figure 4.

3. Effect of temperature on total RNA content per gram of fresh weight:

RNA content of roots grown at different temperatures when

TABLE 1

Effect of temperature and time on the growth of the root of *Pisum sativum* 'Alaska'

Temperature (°C)	24 hr	48 hr	72 hr	Mean	Growth rate (cm/hr)
10°	0.7	1.2	2.2	1.4	0.0305
20°	1.4	3.5	5.5	3.5	0.0786
25°	1.3	3.4	5.1	3.3	0.0710
30°	0.8	2.1	3.5	2.1	0.0458
Mean	1.1	2.6	4.1		

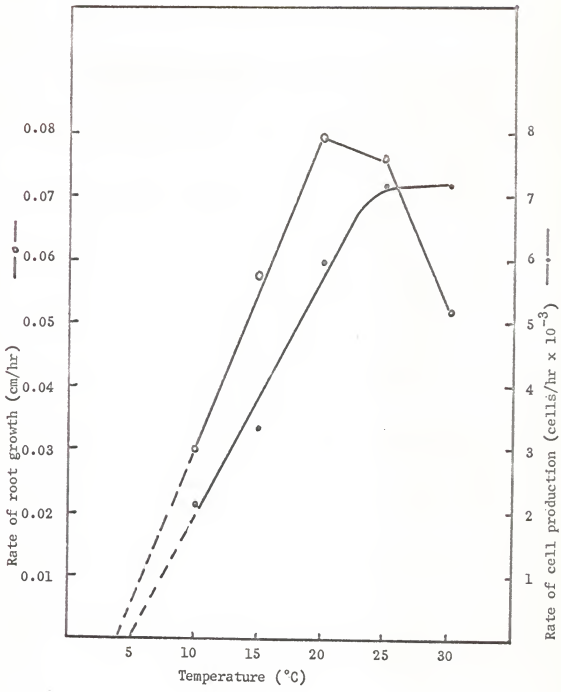


Figure 3

Effect of temperature on rate of cell production and rate of root growth of Pisum sativum 'Alaska'

TABLE 2

Effect of temperature and time on total RNA content of roots (μg/root) of Pisum sativum 'Alaska'

Temperature (°C)	Time				
	0 hr	24 hr	48 hr	72 hr	Mean
10°	64.7	63.96	84.49	91.84	76.25
20°	64.7	75.05	100.41	120.34	90.12
25°	64.7	76.23	98.42	150.09	97.36
30°	64.7	72.23	99.71	104.53	85.29
Mean	64.7	71.87	95.76	116.70	

TABLE 3

Effect of temperature and time on the percentage increase in RNA content of roots of Pisum sativum 'Alaska'

Temperature (°C)	Time			
	24 hr	48 hr	72 hr	Mean
10°	-1.10	30.58	41.94	23.01
20°	16.00	55.19	85.99	52.39
25°	17.80	52.11	131.97	67.29
30°	11.63	54.11	61.56	42.43
Mean	11.36	48.00	80.37	

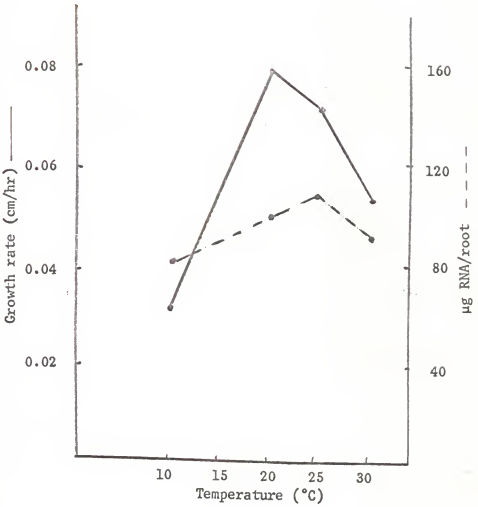


Figure 4

Effect of temperature on growth rate and total RNA content per root of Pisum sativum 'Alaska'

expressed on a fresh weight basis is summarized in Table 4. At 20° and 25° C, time had no effect on total RNA content of roots. There was an increase in RNA at 10° C between 24 and 48 hours but no difference between 0 and 24 or 48 and 72 hours. Between 48 and 72 hours there was a decrease in RNA content in roots grown at 30° C with no differences observed between 0 and 48 hours.

Experiment II: Effect of temperature and time on total RNA content and number of cells in the root tip sections (0.0-1.0 cm):

1. Effect of temperature and time on total RNA content of the 0.0-1.0 cm root tip section:

There were no differences in RNA content in roots grown at 10° and 20° C. A linear decrease was shown in roots grown at 25° C, and a quadratic decrease at 30° C with a large decrease between 0 and 24 hours (Table 5). Roots grown at 10° and 20° C at 72 hours contained more RNA in the root tip section than those grown at 25° and 30° C.

2. Effect of temperature and time on total RNA content per cell of the root tip section:

There was no changes in RNA content per cell in roots grown at 10° C for 72 hours. An increase was observed at 20° C and a linear decrease at 25° and 30° C (Table 6).

3. Effect of temperature and time on number of cells of the root tip section:

The number of cells per root tip section decreased with increase of time from 24 to 48 hours in roots grown at 20° and 25° C. There were no changes in the numbers of cells between 24 and 48 hours in roots grown at 30° C but a decrease was noticed at 72 hours. At 72 hours after temperature treatments roots grown at 20°, 25° and 30° C contained fewer cells than at 24 hours. There were no changes in the numbers of cells in roots grown at 10° C for 72 hours (Table 7).

TABLE 4

Effect of temperature on total RNA content (mg/gr fresh wt) of roots of Pisum sativum 'Alaska'

Temperature (°C)	Time 0 hr	24 hr	48 hr	72 hr	Mean
10°	0.87	0.82	1.03	1.03	0.94
20°	0.87	0.96	0.94	0.99	0.94
25°	0.87	0.86	0.87	0.94	0.88
30°	0.87	0.84	0.85	0.68	0.81
Mean	0.87	0.87	0.89	0.91	

TABLE 5

Effect of temperature and time on RNA content (µg/section) of the 0.0-1.0 cm root tip sections of [illegible] sativum 'Alaska'

Temperature (°C)	Time 0 hr	24 hr	48 hr	72 hr	Mean
10°	24.20	21.89	23.81	20.16	22.52
20°	24.20	21.00	19.20	20.88	21.32
25°	24.20	21.09	15.22	11.24	17.93
30°	24.20	16.79	16.53	11.18	17.18
Mean	24.20	20.19	18.69	15.87	

TABLE 6

Effect of temperature and time on RNA content (10^{-5} X µg/cell) of the 0.0-1.0 cm root tip sections of Pisum sativum 'Alaska'

Temperature (°C)	Time			
	24 hr	48 hr	72 hr	Mean
10°	7.87	7.36	7.36	7.53
20°	7.20	8.34	8.41	7.98
25°	8.10	7.66	5.59	7.12
30°	5.56	5.31	4.72	5.20
Mean	7.18	7.17	6.25	

TABLE 7

Effect of temperature and time on number of cells in the 0.0-1.0 cm (cells/cm section) root tip section of Pisum sativum 'Alaska'

Temperature (°C)	24 hr	48 hr	72 hr	Mean
10°	277,740	270,260	274,000	275,000
20°	291,760	230,240	248,260	256,753
25°	260,260	198,760	201,250	220,090
30°	302,000	311,500	237,000	283,500
Mean	377,253	354,587	320,170	

Experiment III: Effect of temperature on various RNA species in both dividing (R_1) and elongating (R_2) root sections:

1. Fractionation of RNA on a methylated albumin column:

After phenol extraction, RNA was separated on a methylated albumin column. Two fractions of soluble RNA (sRNA-1, sRNA-2), one fraction of DNA-RNA and two fractions of ribosomal RNA (rRNA) were eluted from the column with a linear gradient of NaCl and phosphate buffer at pH 6.7. Figure 5 is a typical plot of the elution pattern of various RNAs. Other elution data are given in tabular form (Table 8). All the fractions eluted from the methylated albumin columns reacted with Orcinol and the DNA-RNA peak was the only fraction that reacted with both Orcinol and diphenylamine.

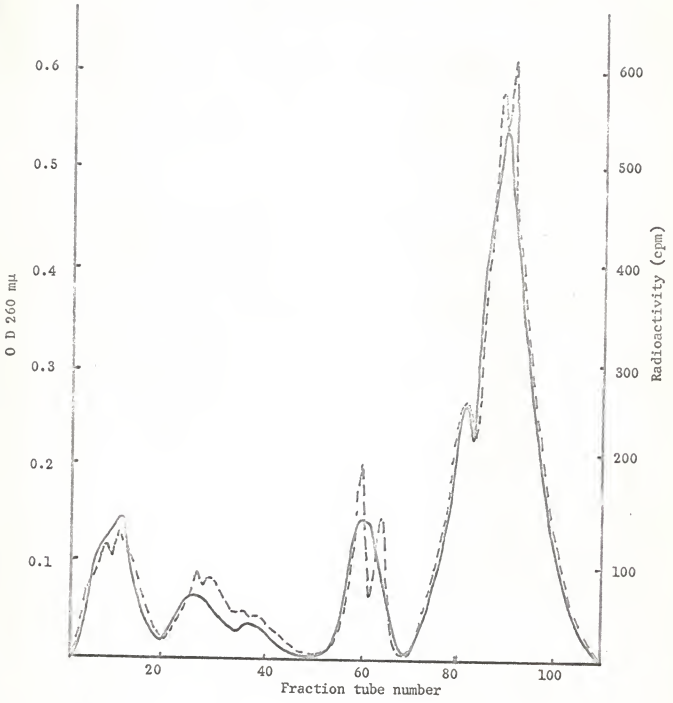
2. Effect of temperature on various RNA species in both R_1 and R_2:

It can be seen in Table 8 that in R_1 an increase in temperature from 5° to 20° C caused a decrease in per cent of sRNA-1 and an increase in rRNA content. No change occurred between 20° and 25° C. Little change was found in the sRNA-2 and DNA-RNA fractions at all temperatures. The ratio between rRNA and sRNA-1 increased with an increase in temperature from 5° to 20° C and decreased with an increase in temperature from 20° to 25° C. This temperature effect on the rRNA/sRNA-1 ratio of the R_1 root tip section was very similar to the effect of temperature on growth rate and mitotic cycle time of pea roots (Figures 6 and 7).

In R_2 there was no difference in sRNA-1 or rRNA/sRNA-1 at any temperature. At 5° C the rRNA content was less and DNA-RNA content was greater than at 10°, 20° or 25° C. Soluble RNA-2 was equal at 5° and 10° C and at 20° and 25° C; the former being greater (Table 8).

In general, an increase in sRNA-1 and a decrease in rRNA was observed when the cell shifted from division (R_1) to elongation (R_2).

Figure 5. Elution pattern of Pisum RNA on a methylated albumin column. Pea seedlings were germinated for 48 hours in vermiculite, transferred to a growth chamber at 20° C for 24 hours and incubated with P^{32} for 15 minutes. Root tips (0.0-0.4 cm) were collected and RNA extracted with phenol. The RNAs were eluted from the column with a linear gradient of NaCl from 0.3 to 1.2 M in 0.05 M phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected. Optical density and radioactivity were determined.


0.6
0.5
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0.3
0.2
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O D 260 mμ
600
500
400
300
200
100
Radioactivity (cpm)
20
40
60
80
100
Fraction tube number

Figure 5

TABLE 8

Effect of temperature on various RNAs (expressed as per cent of total RNA content) in the root section R_1 (0.0-0.4 cm) and R_2 (0.4-1.4 cm) of Pisum sativum 'Alaksa'

	Temperature (°C)	sRNA-1	sRNA-2	DNA-RNA	rRNA	rRNA/sRNA-1
R_1	5°	23.84	9.40	8.13	58.61	2.46
	10°	20.54	7.04	8.50	63.92	2.97
	20°	11.06	7.89	9.41	71.64	6.48
	25°	12.90	8.23	9.47	69.40	5.38
R_2	5°	24.72	12.34	10.53	52.41	2.12
	10°	24.00	12.53	6.79	56.68	2.36
	20°	23.31	9.71	8.45	58.53	2.51
	25°	25.00	9.50	8.81	56.69	2.27

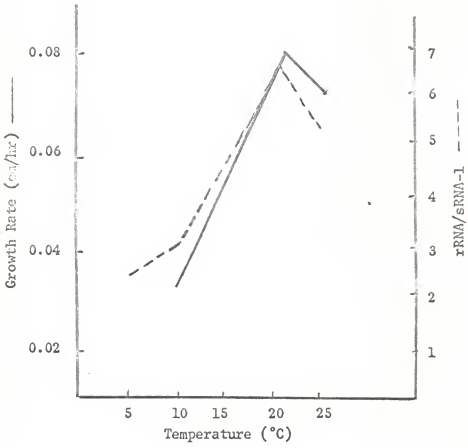


Figure 6

Correlation between temperature rate of root growth and the ratio of ribosomal RNA (rRNA) to soluble RNA-1 (sRNA-1) content of 0.0-0.4 cm root tip sections of Pisum sativum 'Alaska'

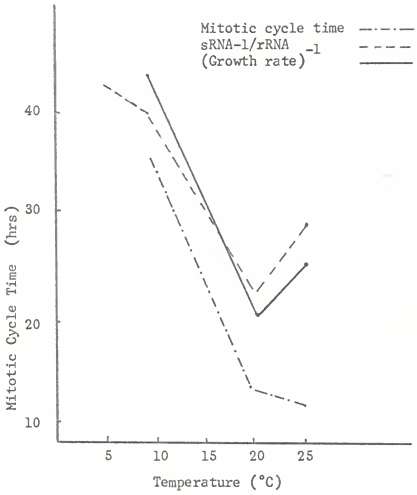


Figure 7

Effect of temperature on growth rate, mitotic cycle time and sRNA-1/rRNA of Pisum sativum 'Alaska'

This was true for all the temperatures tested in this experiment, except at 5° C where no differences were observed in sRNA-1 between R_1 and R_2 (Table 8).

Experiment IV: Effect of temperature on the metabolism of various RNA species:

1. Isotope labeling experiments:

Isotope experiments showed that after 15 or 30 minutes pulse labeling with P^{32}, with both R_1 and R_2, the sRNA-1 fractions eluted from the methylated albumin columns were the only fractions containing radioactivity using roots grown at 10°, 20° and 25° C. The specific activity (counts per minute per optical density unit) of sRNA-1 from pea roots grown at 20° C decreased with an increase in time after pulse labeling in R_1, whereas, in R_2 the specific activity decreased from 0 to 1 hour and then remained constant. The specific activity of sRNA-2, DNA-RNA and rRNA increased with an increase in time after labeling (Table 9).

Pea roots grown at 10° C showed a decreased rate of P^{32} incorporation into the various RNAs except sRNA-1 (Figures 8, 9, 10 and 11) while in roots grown at 20° C a faster incorporation of P^{32} into various RNA fractions was observed.

2. Zone-sedimentation analysis:

After 30 minute pulse labeling of pea roots grown at 10° and 20° C a labeling peak was discovered between 0-5 per cent in the sucrose gradient in R_1 and R_2 root sections. Fractionation of the same material on a methylated albumin column showed a labeling peak in sRNA-1. Four distinct RNA peaks were observed in R_1 root sections grown at 10° and 20° C (Figures 12, 13). In R_2 only three RNA peaks were found in the sedimentation analysis (Figures 14, 15).

TABLE 9

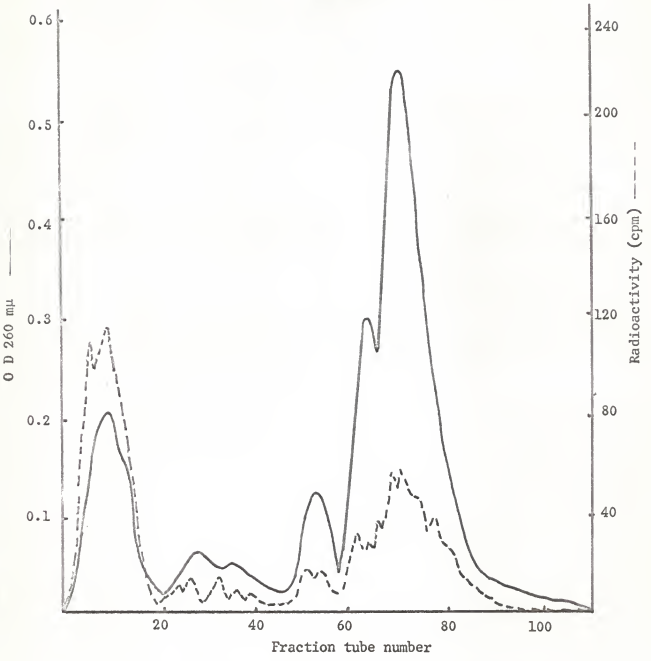
The relationship between specific activity (C P M/O D Unit) of various RNAs eluted from methylated albumin columns and the time after pulse labeling of roots grown at 20° C of Pisum sativum 'Alaska'

Hours after pulse labeling		sRNA-1	sRNA-2	DNA-RNA	rRNA
	.5	157	24	12	13
R_1*	1.0	56	32	27	27
	24.0	67	85	138	133
	.5	103	46	3	14
R_2*	1.0	82	60	21	19
	24.0	88	147	150	120

*R_1 (0.0-0.4 cm) root tip section

*R_2 (0.4-1.4 cm) root section

Figure 8. Elution pattern of Pisum RNA on a methylated albumin column. Pea seedlings were germinated for 48 hours in vermiculite, transferred to a growth chamber at 20° C for 24 hours and incubated with P^{32} for 30 minutes. Root tips (0.0-0.4 cm) were collected and RNA extracted with phenol. The RNAs were eluted from the column with a linear gradient of NaCl from 0.125 to 1.1 M in 0.05 M phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected. Optical density and radioactivity were determined.


0.6
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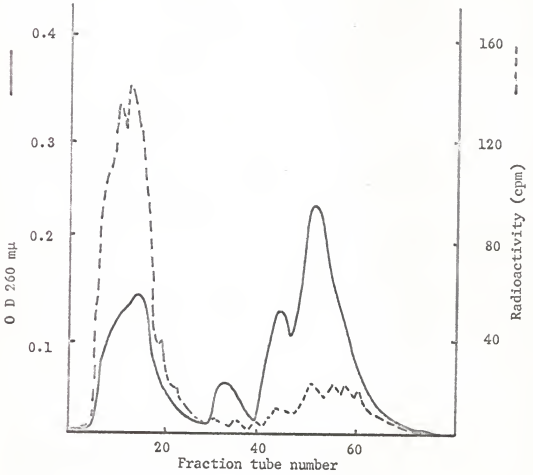
O D 260 mμ ——
240
200
160
120
80
40
Radioactivity (cpm) - - - -
20
40
60
80
100
Fraction tube number

Figure 8

Figure 9. Elution pattern of Pisum RNA on a methylated albumin column. Pea seedlings were germinated for 48 hours in vermiculite, transferred to a growth chamber at 20° C for 24 hours and incubated with P^{32} for 30 minutes. Root sections (0.4-1.4 cm) were collected and RNA extracted with phenol. The RNAs were eluted from the column with a linear gradient of NaCl from 0.125 to 1.1 M in 0.05 M phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected. Optical density and radioactivity were determined.


0.4
0.3
0.2
0.1
O D 260 mμ
160
120
80
40
Radioactivity (cpm)
20
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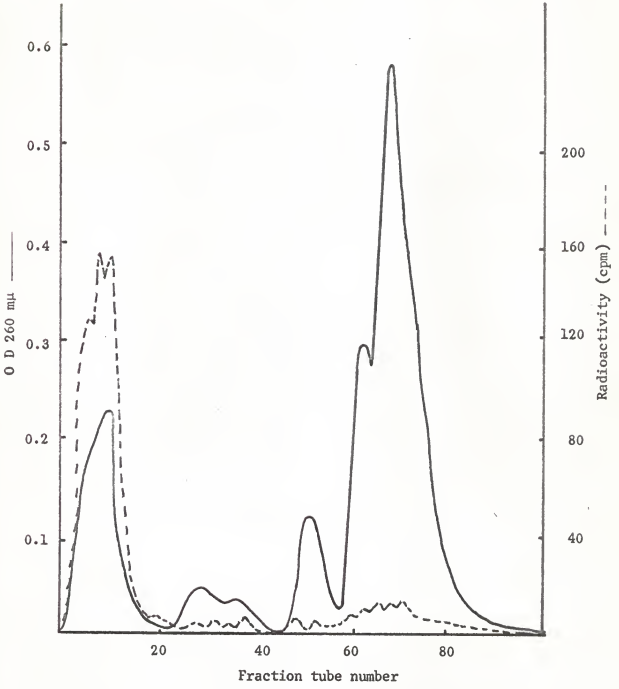
60
Fraction tube number

Figure 9

Figure 10. Elution pattern of Pisum RNA on a methylated albumin column. Pea seedlings were germinated for 48 hours in vermiculite, transferred to a growth chamber at 10° C for 24 hours and incubated with P^{32} for 30 minutes Root tips (0.0-0.4 cm) were collected and RNA extracted with phenol. The RNAs were eluted from the column with a linear gradient of NaCl from 0.125 to 1.1 M in 0.05 M phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected. Optical density and radioactivity were determined.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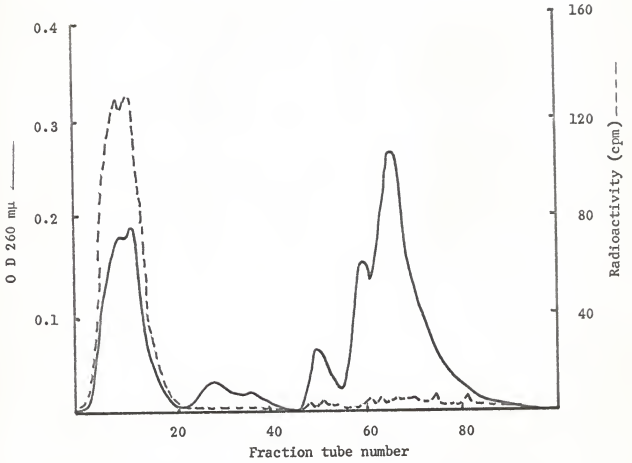
0.2
0.1
O D 260 mμ
200
160
120
80
40
Radioactivity (cpm)
20
40
60
80
Fraction tube number

Figure 10

Figure 11. Elution pattern of Pisum RNA on a methylated albumin column. Pea seedlings were germinated for 48 hours in vermiculite, transferred to a growth chamber at 10° C for 24 hours and incubated with P^{32} for 30 minutes. Root sections (0.4-1.4 cm) were collected and RNA extracted with phenol. The RNAs were eluted from the column with a linear gradient of NaCl from 0.125 to 1.1 M in 0.05 M phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected. Optical density and radioactivity were determined.


0.4
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Radioactivity (cpm)
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Fraction tube number

Figure 11

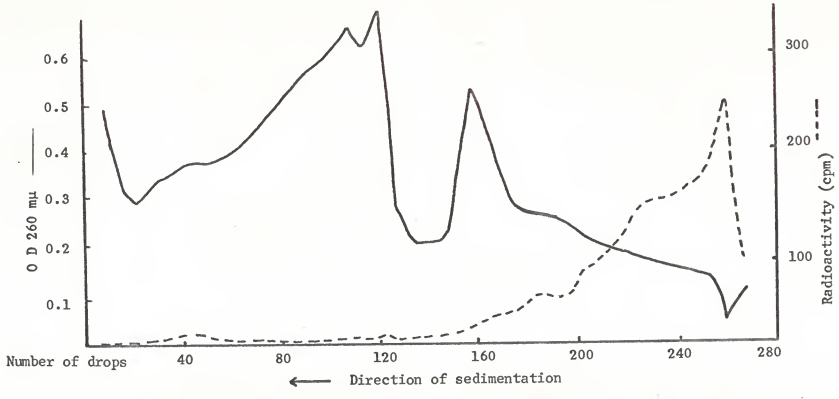

Figure 12

Zone-sedimentation of RNA from Pisum root tip (0.0-0.4 cm) grown at 10° C, labeled with P^{32} for 30 minutes followed by phenol extraction of RNA. Zone-sedimentation analysis was carried out in a sucrose gradient of a 5-25 per cent sucrose centrifuging for 14 hours at 2° C.

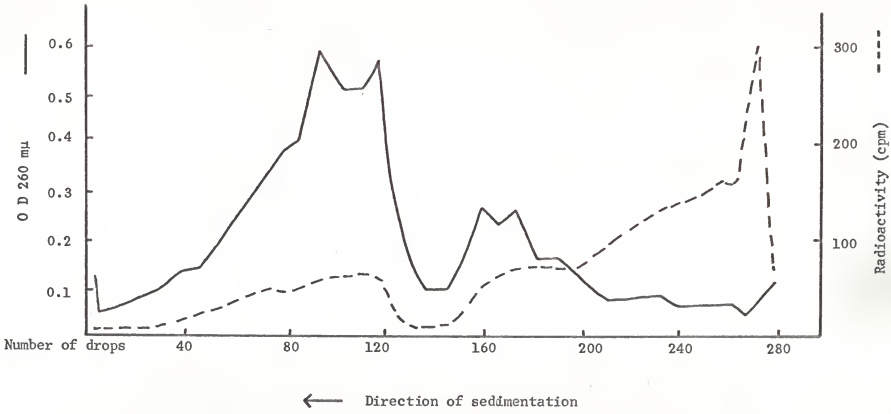

Figure 13

Zone-sedimentation of RNA from Pisum root tip (0.0-0.4 cm) grown at 20° C, labeled with P^{32} for 30 minutes followed by phenol extraction of RNA. Zone-sedimentation analysis was carried out in a sucrose gradient of a 5-25 per cent sucrose centrifuging for 14 hours at 2° C.

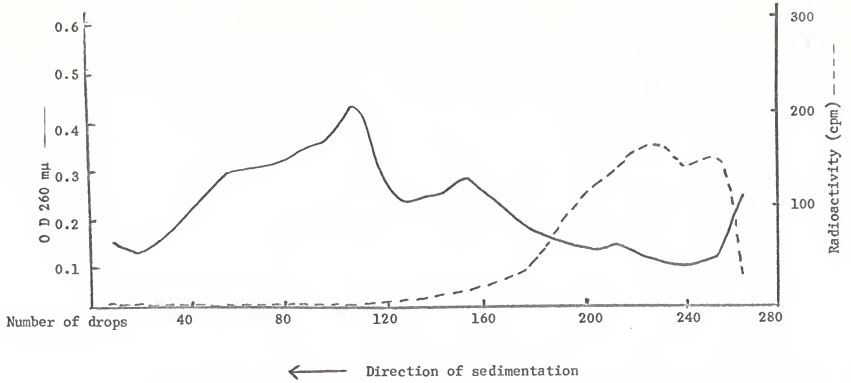

Figure 14

Zone-sedimentation of RNA from Pisum root sections (0.4-1.4 cm) grown at 10° C, labeled with P^{32} for 30 minutes followed by phenol extraction of RNA. Zone-sedimentation analysis was carried out in a sucrose gradient of a 5-25 per cent sucrose centrifuging for 14 hours at 2° C.

Figure 15

Zone-sedimentation of RNA from Pisum root sections (0.4-1.4 cm) grown at 20° C, labeled with P^{32} for 30 minutes followed by phenol extraction of RNA. Zone-sedimentation analysis was carried out in a sucrose gradient of a 5-25 per cent sucrose centrifuging for 14 hours at 2° C.

DISCUSSION

In pea roots a close simularity between the Q_{10} of respiration, chemical reactions and cell production has been shown in previous reports (68, 74). However, a different relationship has been found between the Q_{10} for gorwth rate and respiration (68). This indicates that cell production is controlled by chemical reactions, while cell elongation is affected by something more than a simple chemical phenomenon. The rate of cell production did not parallel with the rate of root growth (Figure 3). The difference could be due to rate of cell expansion.

In experiment I, total RNA content of roots grown at 25° C increased rapidly between 48 and 72 hours (Table 2 and 3) because of the initiation of secondary roots. Total RNA content per root increased with an increase of root length.

Woodstock and Skoog (78) showed a positive correlation in the RNA content of six strains of corn and their growth rates. The data from experiment II indicated that the total RNA content of the 1 cm root tip sections using temperature as a variable was not correlated with growth rate. The conclusion can then be made that growth rate was not necessarily determined by the RNA content of the root tip section. This has also been suggested by Ingle and Hageman (29).

The decrease with time of RNA content of the 1 cm root tip section of roots grown at 20° and 25° C may be due to the increase in ribonuclease activity as the root matured. Evidence that this phenomenon occurred has been shown by other workers (2, 47). The suggestion has been made

that RNA is metabolized by the enzyme and thus is made available for growth. In experiment II there was no change in RNA content of the 1 cm root tip section when the roots were grown at 10° C. However, a decrease was noticed in roots grown at 30° C. These results suggest that at temperature below optimum for growth, although RNA content was higher, it is not available for growth. A decrease in growth of plants grown at temperatures higher than optimum may be due to a decrease in RNA content possibly because of a breakdown of RNA previously deposited in the root tip or because of rapid utilization.

In experiment III the elution pattern of RNA of pea roots from methylated albumin columns was similar to that of peanut cotyledons reported by Cherry et al. (12) except that the mRNA associated with the heavy ribosomal fraction was not detected. This may be due to the differences in plant material or to difference in the half life of mRNA for pea roots and peanut cotyledons. All fractions eluted from the methylated albumin column reacted with Orcinol which indicates they are RNAs. The DNA-RNA fraction reacted with both Orcinol and diphenylamine suggesting that this fraction contained both DNA and RNA. The elution pattern of this DNA-RNA fraction was similar to that of Cherry's DNA-RNA complex fraction. Studies and characterizations of this fraction in peanut cotyledons have been carried out by Cherry (11).

In R_1, the sRNA-1 and rRNA fractions comprised a different proportion of the total RNA at different growth rates. The ratio of these two fractions showed a correlation with both growth rate and mitotic cycle time in pea roots grown at different temperatures (Figures 6, 7). In R_2, there was no relationship between various RNA species content and growth rate as affected by temperature. These data support

the hypothesis that only meristematic cells can the synthesis of sRNA-1 and rRNA be regulated by temperature treatment and thus control the rate of root growth.

Section R_2 contained more sRNA-1 and less rRNA and R_1 at 10°, 20° and 25° C. Heyes (24) reported a decrease in RNA_1 with an increase in RNA_2 as the cells underwent expansion. He stated that RNA_1 is in the form of microsome and explained that this change in RNA_1 and RNA_2 was due to an increase in ribonuclease activity, leading to a disruption of the microsomes and an accumulation of RNA_2. The RNA_2 does not attach to a particular protein, thus causing the cells to shift from division to elongation. The results of this experiment suggested that the decrease in Heyes' RNA_1 represented the decrease of rRNA and the increase in RNA_2, the sRNA-1.

The fact that different fractions of RNA in R_2 were not altered by temperature treatments suggested that if cell expansion is related to RNA, it is because of RNA base composition rather than quantity.

In all of the pulse labeling experiments the sRNA-1 fraction was the only fraction found containing radioactivity. This suggested that the sRNA-1 fraction was rapidly metabolized and the rate of P^{32} incorporation was not affected by temperature or cell type (dividing or elongating).

When temperature decreased from 20° to 10° C there was a decrease in the rate of P^{32} incorporation into the various RNA fractions except sRNA-1 (Figures 7, 8, 9, 10). This indication that the corresponding decrease in growth and lengthening of the mitotic cycle time with decreases of temperature from 20° to 10° C was partially a result of a decreasing rate of the synthesis of the other various RNAs.

Characterization of sRNA-1 was carried out by zone sedimentation

analysis and methylated albumin column fractionation of a 30 minute pulse labeled R_1 and R_2 pea root sections. Results of these studies indicated that this rapidly metabolized sRNA-1 had a sedimentation coefficient of four or less. In HeLa cells a 30 minute incubation in tritiated cytidine medium resulted in essentially exclusive labeling of nuclear RNA (49). Sedimentation analysis of cells labeled in this way showed a labeled peak at 4s (50). In isolated pea nuclei, low molecular weight RNA with the characteristics of amino acid transfer RNA was synthesized (13). Therefore, this RNA fraction may be of nuclear origin with the ability to transfer amino acids to the ribosomal template. Cherry et al. (12) suggested that this rapidly labeled sRNA fraction may be partially degraded products or imcomplete molecules of heavy ribosomal and messenger RNAs. Pulse labeling data presented here indicated a decrease in specific activity of sRNA-1 with an increase in time after labeling (Table 9) which suggests that this sRNA-1 fraction may be a complex of sRNA, mRNA and precursors of rRNA.

Studies of the regulation of RNA synthesis have only recently been conducted in some laboratories and most of the information has been obtained from microorganisms. Contradictory results have been reported (34, 36, 43, 44, 57). Now it is known that only certain portions of the ribosomes in the cell are capable of protein synthesis, depending on the presence or absence of mRNA. The amino acid requirement for RNA synthesis has been reported by Gros and Gros (18) and Pardee and Prestidge (48). Neidhardt (43) has shown that the synthesis of a special protein is involved in the regulation of rRNA synthesis. Under normal growth conditions sRNA-1 and rRNA represent 80-90 per cent of the total cellular RNA. Since both are involved in protein synthesis, the changes

in sRNA-1 and rRNA in the root meristematic cells with the change in growth rate may be mediated by the effect of the synthesis of mRNA and thus, the synthesis of special proteins. If this is so, then the proposed model of Jacob and Monod (31) could be used to interpret this regulating mechanism. In Jacob-Monod's model the basic elements of the control system are: (1) a structural gene, (2) a regulator gene and (3) an operator gene. The structural gene produces a mRNA molecule which serves as the template for protein or enzyme synthesis. The regulator gene produces a repressor RNA molecule which can interact with the operator gene. Combination of the repressor and operator genes prevent the structural gene from making mRNA. In the same way, the different levels of sRNA-1 and rRNA observed at different temperatures may affect the production of mRNA, thus acting as a modifying influence at the level of protein biosynthesis.

SUMMARY

The effect of temperature on growth and the metabolism of ribonucleic acid in relation to cell division and cell elongation of Pisum sativum 'Alaska' has been studied.

The rate of pea root growth was temperature dependent. The rate of root growth was greatest at 20° and 25° C. The effect of temperature on the rate of cell production was not parellel with the rate of cell expansion.

The effect of temperature on total RNA content in the 1 cm root tip section was not correlated with the growth rate which suggests that growth rate was not necessarily determined by RNA content of the root tip section. A decrease in total RNA content of the root tip section with an increase in time was also shown.

The number of cells in the root tip section decreased with an increase in time except at 10° C.

The effect of temperature on the metabolism of various RNA species has been studied by separating phenol extracted RNA on methylated albumin columns. The nucleic acid extract was separated into five fractions; sRNA-1, sRNA-2, one DNA-RNA and two rRNAs. In R_1 (0.0-0.4 cm root tip section) an increase in temperature from 5° to 20° C caused a decrease in sRNA and an increase in rRNA content. In R_2 (0.4-1.4 cm) various RNA species were not affected by temperatures. A close relationship between temperature, growth rate, mitotic cycle time and sRNA-1/rRNA ratio in R_1 was shown. The proposed hypothesis is that in higher plants

only in the meristematic cells can the synthesis of RNA be regulated by temperature treatment, and thus control the rate of growth. Results of these studies have shown there was an increase in sRNA-1 with a decrease in rRNA which resulted in the synthesis of particular proteins and the cells shifted from division to elongation.

The effect of the decrease in temperature from 20° to 10° C on the corresponding decrease in growth and lengthening in mitotic cycle time was partially a result of decreasing rate in synthesis of various RNAs.

Isotope labeling experiments and zone-sedimentation analysis indicated that sRNA-1 is rapidly metabolized with a sedimentation coefficient of four or less. Pulse labeling experiments furnished evidence to show that the specific activity of sRNA-1 decreased with an increase in time after labeling, while the specific activity of sRNA-2, DNA-RNA and rRNA increased with increase in time after labeling, suggesting that this sRNA-1 may be a complex of sRNA, mRNA and precursor of rRNA.

Jacob-Monods' model of the regulation of protein synthesis has been used to discuss the possible regulating mechanism of RNA in pea roots grown at different temperatures.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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